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CERTIFICATION REPORT

The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (Fucus vesiculosus): ERM®- CD200

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The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (Fucus vesiculosus): ERM®- CD200

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Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM-CD200, a powdered bladderwrack material (species *Fucus vesiculosus*) certified for the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn. The material was produced following ISO Guide 34:2009 [1].

Approximately 60 kg of brown algae seaweed (bladderwrack, *Fucus vesiculosus*) was collected in Galway (Ireland) and processed at IRMM (Belgium) to produce a certified reference material (CRM) of seaweed powder. The produced vials containing the processed seaweed were carefully capped, sealed and stored for further certification studies.

Between-unit homogeneity was quantified as well as stability during dispatch and storage in accordance with ISO Guide 35:2006 [2]. Within-unit homogeneity was also quantified to determine the minimum sample intake.

The material was characterised by an inter-laboratory comparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [3] including uncertainty contribution related to possible heterogeneity and instability of the material as well as to the characterisation.

The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies.

The CRM is available in amber glass vials containing approximately 5 g of dried powder, placed in aluminized polythene sachet closed under a nitrogen atmosphere.

The minimum amount of sample to be used is 200 mg.

The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

The following values were assigned:

Certified values

	Mass fraction	
	Certified value [1,2)] [mg/kg]	Uncertainty [3)] [mg/kg]
As	55	4
Cd	0.95	0.06
Cu	1.71	0.18
Hg	0.0186	0.0016
Pb	0.51	0.06
Se	0.088	0.010
Zn	25.3	1.7

1) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified value and its uncertainty are traceable to the International System of Units (SI).

2) Certified mass fractions are corrected for the water content of the material (and expressed as dry mass), determined as described in the section "Instructions for use".

3) The uncertainty is expanded with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

1 Table of content

2 Glossary

AAS	Atomic absorption spectrometry
AFS	Atomic fluorescence spectrometry
ANOVA	Analysis of variance
b	Slope in the equation of linear regression $y = a + bx$
BCR®	Trademarks of CRMs owned by the European Commission; formerly Community Bureau of Reference
CI	Confidence interval
CRM	Certified reference material
CV-AAS	Cold vapour atomic absorption spectrometry
CV-AFS	Cold vapour atomic fluorescence spectrometry
DMA	Direct Mercury Analyzer
EC	European Commission
ERM®	Trademark of European Reference Materials
EU	European Union
ET-AAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
GUM	Guide to the Expression of Uncertainty in Measurements *[ISO/IEC Guide 98-3:2008]*
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-SFMS	ICP-Sector-field mass spectrometry
ID	Isotope dilution
(I)NAA	(Instrumental) neutron activation analysis
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
LOD	Limit of detection
k	Coverage factor
k_0-NAA	k_0-Neutron Activation Analysis
KFT	Karl Fischer titration
MS	Mass spectrometry
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
MW	Microwave
n	Number of replicates per unit
N	Number of samples (units) analysed
n.a.	Not applicable
n.c.	Not calculated
OES	Optical emission spectrometry
p	Number of independent observations
PSA	Particle size analysis
PT	Proficiency testing
QC	Quality control
rel	Index for 'relative'
RM	Reference material
RSD	Relative standard deviation
r^2	Coefficient of determination of the linear regression
s	Standard deviation
s_{bb}	Between-unit standard deviation
$s_{between}$	Standard deviation between groups as obtained from ANOVA
SF-MS	Sector-field mass spectrometry
SI	International System of Units

s_{meas}	Standard deviation of measurement data
s_{within}	Standard deviation within groups as obtained from ANOVA
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
t_i	Time point for each replicate
t_{sl}	Proposed shelf life
u	Standard uncertainty
U	Expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit heterogeneity that could be hidden by method repeatability
u_{bb}	Standard uncertainty related to a possible between-unit heterogeneity
u_c	Combined standard uncertainty
u_{cal}	Standard uncertainty of calibration
u_{char}	Standard uncertainty of the material characterisation
u_{CRM}	Combined standard uncertainty of the certified value
U_{CRM}	Expanded uncertainty of the certified value
u_Δ	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability
u_m	Standard uncertainty of the measurement
U_{meas}	Expanded uncertainty of the measurement
u_{rec}	Standard uncertainty related to possible between-unit heterogeneity modelled as rectangular distribution
u_{sts}	Standard uncertainty of the short-term stability
UV	Ultraviolet
V	Volume
Vis	Visible
WFD	Water Framework Directive
$\bar{x}$	Arithmetic mean
α	significance level
Δm	Absolute difference between mean measured value and the certified value
ν_{smeas}	Degrees of freedom for the determination of the standard deviation s_{meas}
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background: need for the CRM

The certified reference material ERM-CD200 has been developed to satisfy the demands of laboratories dealing with environmental and food safety analyses. In particular, the European Water Framework Directive 2000/60/EC, although not providing metal concentration limits in algae, suggests the use of (macro)algae as an indicator of water quality [4]. ERM-CD200 is intended mainly for verification of the accuracy of trace element measurement in algae and similar matrices.

1.2 Choice of the material

Among the three groups of algae (red, green, brown), brown algae demonstrated to be an efficient class of biosorbents, especially for Cd, Cu, Zn, Pb, Cr and Hg in water and wastewater; due to their higher uptake capacity compared to red and green algae.

Fucus vesiculosus together with *Ascophillum nodosus* are increasingly used for the purpose of environmental monitoring, especially for water quality control [5]. Some European countries (mainly Finland, Sweden, Germany and the UK) used macroalgae (and in particular *Fucus vesiculosus*) as a marine bioindicator.

Furthermore, brown algae are used in the preparation of foods, cosmetics and medicines for their nutritional and therapeutic properties, which makes them of interest to analysts and scientists concerned with monitoring levels of trace elements [6].

1.3 Design of the project

ERM-CD200 has been certified for its total content of As, Cd, Cu, Hg, Pb, Se and Zn through the results of an inter-laboratory comparison.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel (BE)

(accredited to ISO Guide 34 for production of certified reference materials, BELAC No 268-RM)

2.2 Collection of material and Processing

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE)

(accredited to ISO Guide 34 for production of certified reference materials, BELAC No 268-RM)

Martin Ryan Institute, Galway, IE)

2.3 Homogeneity study

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE)

(accredited to ISO Guide 34 for production of certified reference materials, BELAC No 268-RM)

ALS Scandinavia AB, Luleå, SE)

(Measurements performed under the scope of ISO/IEC 17025 accreditation, SWEDAC-1087)

2.4 Stability study (alphabetic order)

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel (BE)

(accredited to ISO Guide 34 for production of certified reference materials, BELAC No 268-RM)

ALS Scandinavia AB, Luleå (SE)

(Measurements performed under ISO/IEC 17025 accreditation; SWEDAC 1087)

ChemService S.r.l. Controlli e ricerche, Milano (IT)

(Measurements performed under the scope of ISO/IEC 17025 accreditation; SINAL-0004)

Umweltbundesamt, Umweltanalytik & Chemikalien, Wien (AT)

(Measurements performed under the scope of ISO/IEC 17025 accreditation, BMWA-92.714/0518-I)

2.5 Characterisation (alphabetic order)

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel (BE)

(accredited to ISO Guide 34 for production of certified reference materials, BELAC No 268-RM)

ALS Scandinavia AB, Luleå (SE)

(Measurements performed under the scope of ISO/IEC 17025 accreditation, SWEDAC-2030)

Ceinal, S.A. (Silliker), Área Análisis Físico-Químicos, Barcelona (ES)

(Measurements performed under ISO/IEC 17025 accreditation; ENAC 257/LE413)

Centre for Environment, Fisheries and Aquaculture Science, Lowestoft (UK)

(Measurements performed under ISO/IEC 17025 accreditation; UKAS 1875)

Comisión Nacional de Energía Atómica (CNEA), Bariloche, Laboratorio de Análisis por Activación Neutrónica, Argentina (AR)

Fødevareinstituttet, Danmarks Tekniske Universitet, Søborg (DK)

(Measurements performed under the scope of ISO/IEC 17025 accreditation, DANAK-350)

The Food and Environment Research Agency, York (UK)

(Measurements performed under ISO/IEC 17025 accreditation; UKAS 1642)

Institut "Jozef Stefan" (IJS), Department of Environmental Sciences, Ljubljana (SI)

(Measurements performed under the scope of ISO/IEC 17025 accreditation, Slovenka Akreditacija-LP090)

INRIM – Unità di Radiochimica e Spettroscopia, Dipartimento di Chimica, Pavia (IT)

MTT – Agrifood Research Finland, Jokioinen (FI)

(Measurements performed under the scope of ISO/IEC 17025 accreditation, FINAS-T024)

Studiecentrum voor Kernenergie (SCK), Mol (BE)

(Measurements performed under the scope of under ISO/IEC 17025 accreditation; BELAC, 015-TEST)

TÜBITAK – UME, Gebze Yerleşkesi, Gebze/Kocaeli, Turkey (TR)

Umweltbundesamt, Umweltanalytik & Chemikalien, Wien (AT)

(Measurements performed under the scope of ISO/IEC 17025 accreditation, BMWA-92.714/0518-I)

Vlaamse instelling voor technologisch onderzoek (VITO), Mol (BE)

(Measurements performed under the scope of ISO/IEC 17025 accreditation; BELAC, 045-TEST)

2.6 Additional material information

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE

(accredited to ISO Guide 34 for production of certified reference materials, BELAC No 268-RM)

Sympatec Benelux, Clausthal-Zellerfeld, DE

3 Material processing and process control

3.1 Origin and processing of the material

The material used for the production of ERM-CD200 is brown algae bladderwrack (*Fucus vesiculosus*), a large olive-brown coloured seaweed, which can grow up to 2m in length. It can be found on the middle-shore, where it grows attached to rocky substrates, often associated with knotted wrack (*Ascophyllum nodosum*). *Fucus vesiculosus* occurs around the coastline of Britain, Ireland, the Baltic Sea, Norway, the Atlantic coast of France, Spain and Morocco, as well as Greenland, and the eastern coasts of Canada and the USA.

The raw material used for the production of ERM-CD200 originates from Galway, in Ireland.

Sixty kg of brown algae seaweed were collected at two sites, in Silver Strand beach and Spiddal (IE). Plants were cut above the holdfast by using a sharp knife, immediately rinsed with seawater to remove debris and sand and then collected in bags. In the laboratory, plants were checked for large epiphytes and other animal material, briefly rinsed in freshwater and immediately frozen at - 20 °C.

Afterwards, the frozen raw material was transported to the IRMM (BE) in order to be processed under controlled humidity and temperature conditions.

At the processing sector of IRMM (BE), the frozen algae was slowly defrosted at room temperature, washed with deionised water to eliminate excess sand, dried in a drying cabinet (Elbanton, Kerkdriel, NL) at 25 ± 5 °C and milled with a cryo-grinding vibrating mill (Palla mill, KHD, Humboldt-Wedag**,** Köln, DE) which was previously cooled to about -190 °C. The dried material was sieved at 125 µm and finally mixed for several hours using a DynaMix CM200 mixer (WAB, Basel, CH). The mixed powder was filled into 5 g portions into acid washed 30 mL amber glass bottles using an automatic filling machine (Allfill, Sandy, UK). Bottles were then labelled and capped using an assembly from Bausch & Ströbel (Ilshofen, DE) and BBK (Beerfelden, DE).

3.2 Process control

Particle size distribution was checked from the beginning to the end of the processing by using a Sympatec Helos particle analyser (Clausthal-Zellerfeld, DE) on randomly selected units. The resulting material showed an average particle size of ca. 100 µm (Figure 1).
An inter-comparison study on PSA was also performed and details are given in section 7.3.

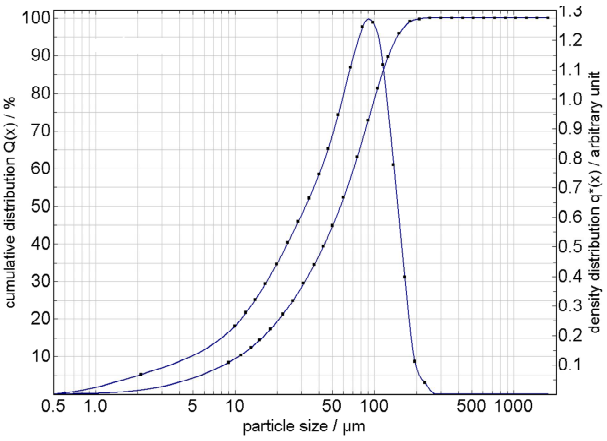

Figure 1: Average particle size distribution obtained using 2-propanol as dispersant (3 replicates)

The moisture level was decreased to a final value of 2.5 % by vacuum drying the milled algae before bottling. A batch of about 1200 vials (units) was then produced and each unit was placed in aluminized polythene sachets flushed with nitrogen gas.

All units were sterilised by gamma irradiation (Isotron B.V., NL) with a minimum radiation dose of 15 kGy.

4 Assessment of homogeneity

A key requirement for any reference material is the equivalence between the various units. In this respect, it is not relevant whether the variation between units is significant compared to the analytical variation, but whether this variation is significant to the certified uncertainty. Consequently, ISO Guide 34 requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit homogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit homogeneity is therefore necessary to determine the minimum sample intake.

'Unit' is defined as an individual glass bottle of ERM-CD200.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all 1200 units, within the stated uncertainty.

For the between-unit homogeneity test, 11 units were selected using a random stratified sampling scheme covering the whole batch, which was divided into 11 groups (with about 110 units per group). One unit was randomly selected from each group. Three independent aliquots were taken from each unit and were digested in concentrated HNO_3 at elevated temperature and pressure, using a microwave apparatus. All elements were measured by ICP-SFMS. The measurements were performed under repeatability conditions, and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. The results were corrected for the water content determined in each bottle number (see section 9.5). All results are reported in Annex A.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling or analytical sequences were detectable for any of the measured elements at the 99 % confidence level. The datasets were also tested for consistency using Grubbs outlier tests on a confidence level of 99 % on individual results and unit means. Some outlying unit means were detected. However, as no technical reason for the outliers could be identified, all data were retained for the final statistical analysis.

Quantification of between-unit heterogeneity was done by analysis of variance (ANOVA), which can separate the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual aliquots are representative for the whole unit.

Evaluation by ANOVA requires that the unit means should follow at least a unimodal distribution and results for each unit should also follow at least unimodal distribution with approximately the same standard deviations. Minor deviations of the individual values from

unimodality do not affect the estimate of between-unit standard deviations. Distribution of the unit averages was tested using histograms and normal probability plots. The results of all statistical evaluations are given in Table 1.

Table 1: Results of the homogeneity study for ERM-CD200

Element	Trends		Outliers		Distribution	
	Analytical sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
As	no	no	-	1	unimodal	unimodal
Cd	no	no	-	-	unimodal	unimodal
Cu	no	no	-	1	normal	unimodal
Hg	no	no	-	-	normal	normal
Pb	no	no	-	-	normal	normal
Se	no	no	-	-	normal	unimodal
Zn	no	no	-	-	normal	normal

Method repeatability ($s_{wb,rel}$), between–unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}}$$

$s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and are therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum heterogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [7]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}}\sqrt[4]{\frac{2}{v_{MSwithin}}}}{\bar{y}}$$

MS_{within} mean square within a unit from an ANOVA

$MS_{between}$: mean squares between-unit from an ANOVA

$\bar{y}$ average of all results of the homogeneity study

n: average number of replicates per unit

$v_{MSwithin}$: degrees of freedom of MS_{within}

A different approach was adopted for As and Cu for which outlying unit averages were detected. In these cases, as the presence of outlying individual values do not exactly reflects the real distribution of these elements in the material, between-unit heterogeneity was modelled as a rectangular distribution limited by the largest outlying unit average, and the rectangular standard uncertainty of homogeneity was estimated by

$$u_{rec,rel} = \frac{\left|outlier - \bar{y}\right|}{\sqrt{3} \cdot \bar{y}}$$

where:

$\bar{y}$ = average of all results of the homogeneity study

The results of the evaluation of the between-unit variation are summarised in Table 2.

Table 2: Results of the homogeneity study; n.c.: cannot be calculated as $MS_{between} < MS_{within}$ n.a.: not applicable

Element	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rec,rel}$ [%]	$u_{bb,rel}$ [%]
As	1.95	1.55	0.62	2.83	2.83
Cd	1.66	n.c.	0.53	n.a.	0.53
Cu	2.57	1.97	0.81	3.74	3.74
Hg	3.64	2.55	1.16	n.a.	2.55
Pb	2.88	1.99	0.91	n.a.	1.99
Se	4.58	2.31	1.45	n.a.	2.31
Zn	2.45	0.92	0.78	n.a.	0.92

The homogeneity study showed no trends in the filling sequence and no outlying unit means for Cd, Hg, Pb, Se and Zn. Thus, the between-unit standard deviation for these elements can be used as estimate of u_{bb}. As u^*_{bb} sets the limit of the detection for the homogeneity study, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential heterogeneity.

Two outlying unit means were found for As and Cu and the inhomogeneity was quantified as u_{rec}, taking the outlying values into account. For these elements, u_{rec} was used as estimate of u_{bb}. When combined as described in section 7.1 to give the uncertainties on the certified values, U_{CRM}, these were still found to be suitably low to allow the material to be used as intended, for method validation.

4.2 Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. Due to the intrinsic heterogeneity, individual aliquots of a material will not contain the same amount of analyte. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the certified value within its stated uncertainty.

In this study, the minimum sample intake was assessed by using the method information supplied by the laboratories participating in the characterisation study. The smallest sample intake that still yielded results with acceptable trueness to be included in the respective studies was taken as minimum sample intake. Almost half the laboratories used a sample intake of between 200 and 300 mg. For each set of measurements from these laboratories, the measurement RSD lay well below the assigned $U_{CRM,rel}$ for each of the elements. Therefore the minimum sample intake is set to 200 mg for all elements.

For Hg, one laboratory used a technique with a mean sample intake of 36 mg. This returned measurement RSD of 7 %, in comparison with the $U_{CRM,rel}$ of 8 %. This indicates that sample intakes of this level may be suitable for Hg measurement. However, as this could not be confirmed by measurement with other techniques, the minimum sample intake for Hg was also set to 200 mg.

5 Stability

Stability testing is necessary to establish conditions for dispatching ERM-CD200 to customers (short-term stability) as well as conditions for storage (long-term stability).

Time, temperature and light were regarded as the most relevant influences on stability of the materials. The influence of light was minimised by the choice of the container that eliminates most of the incoming light (i.e. amber bottles placed in aluminized sachet). In addition, materials are stored and dispatched in the dark, thus practically eliminating the possibility of degradation by light. Therefore, only the influences of time and temperature needed to be investigated. During transport, especially in summer time, temperatures up to 60 °C could be reached and stability under these conditions must be demonstrated if transport at ambient temperature will be applied.

The stability studies for ERM-CD200 were carried out using an isochronous design [8]. In this approach, selected units are stored for a certain time at different temperature conditions. Afterwards, the units are moved to conditions where further degradation can be assumed to be negligible ("reference conditions"), effectively "freezing" the degradation status of the materials. At the end of the isochronous storage, the units are analysed simultaneously under repeatability conditions; improving in this way the sensitivity of the stability tests.

5.1 Short-term stability study

A specific number of units were selected using a random stratified sampling scheme from the whole batch produced and stored for time periods of 0, 1, 2 and 4 weeks at two different temperatures, 18 °C and 60 °C (test conditions). The reference temperature was set to -20°C. Two units were stored for each time/temperature combination, with the exception of time point 0 for which only 2 bottles were set aside. The total number of units reserved for this study was 14. All units were analysed in triplicate at the end of the study providing a total of 6 results per time/temperature combination. Each unit was digested by using a mixture of HNO_3 and HCl for As, Cd, Cu, Pb and Zn or HNO_3, H_2O_2 and HF for Hg and Se. The measurements were performed by means of an ICP-OES for As, Cd, Cu, Pb and Zn and ETAAS or CV-AAS for Se and Hg, respectively. The measurements were performed under repeatability conditions, and in a randomised manner to be able to separate a potential analytical drift from a trend over storage time. The results were corrected for the water content determined in each unit by using the procedure in section 9.5.

The obtained data were evaluated individually for each temperature (18 °C and 60 °C). The results were screened for outliers using the single and double Grubbs test. No outlying values were evidenced by single or double Grubbs test at the 99 % confidence level. Regression lines of mass fraction against storage time were calculated. The slopes were

then tested for statistical significance (which indicates loss/increase due to shipping conditions). In all cases the slopes of the regression lines were not significantly different from zero at 18 °C and 60 °C.

The results of the measurements are shown in Annex B, while the results of the statistical evaluation of the short-term stability are summarised in Table 3.

Table 3: Results of the short-term stability tests

Element	Number of individual outlying results		Significance of the trend at a 99% confidence level	
	18 ºC	60 ºC	18 ºC	60 ºC
As	none	none	no	no
Cd	none	none	no	no
Cu	none	none	no	no
Hg	none	none	no	no
Pb	none	none	no	no
Se	none	none	no	no
Zn	none	none	no	no

No outliers were observed and no trend was statistically significant at a 99 % confidence level for any of the temperatures. As the material showed no sign of degradation at 60 ºC, it may be transported at ambient conditions without further precautions.

5.2 Long-term stability study

For the long-term stability study, units were selected from the whole batch produced by a random stratified sampling scheme and stored for 0, 8, 16 and 24 months at 18 °C. The reference temperature was set to -20 °C. Two units were stored for each time/temperature combination, with the exception of 2 units for time point 0. From each unit, three aliquots were digested in HNO_3 and H_2O_2 using a microwave system. Measurements were performed under repeatability conditions using ICP-MS, and in a randomised manner to be able to separate a potential analytical drift from a trend over storage time. The results were corrected for the water content determined in each unit as described in section 9.5.

The data obtained were evaluated and screened for outliers using the single and double Grubbs test, and no outlying individual results were found significant at a 99 % confidence interval (Table 4). Regression lines of mass fraction against storage time were calculated. The slopes of the regression lines were then tested for statistical significance (which indicates loss/increase due to storage conditions). For all elements, the slopes of the regression lines were not significantly different from 0 (on 99 % confidence level) for the 18 °C test condition.

The results of the measurements are shown in Annex C, and the results of the statistical evaluation of the long-term stability study are summarised in Table 4.

Table 4: Results of the long-term stability tests for ERM-CD200

Element	Number of individual outlying results	Significance of the trend on a 99% confidence level
	18 ºC	18 ºC
As	none	no
Cd	none	no
Cu	none	no
Hg	none	no
Pb	none	no
Se	none	no
Zn	none	no

No technically unexplained outliers were observed and none of the trends was statistically significant at a 99 % confidence level for any of the temperatures.
The material can therefore be stored at 18 ± 5 °C.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can rule out degradation of materials completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time".

Uncertainties of stability during dispatch and storage were estimated as described in [9] for each element. In this approach, the uncertainty of the linear regression line with a slope of zero is calculated and the uncertainty contributions u_{sts} and u_{lts} are then estimated as the product of the chosen shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{tt}$$

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot t_{sl}$$

RSD relative standard deviation of all results of the stability study

x_i result at time point *i*

$\bar{x}$ mean results for all time points

t_{tt} chosen transport time (1 week at 60 ºC)

t_{sl} chosen shelf life (24 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch was estimated from the 60 °C studies for a time of 0.25 months (1 week). The uncertainty therefore describes the possible change during a dispatch at 60 °C lasting for one week.

- $u_{lts,rel}$, the uncertainty of the stability during storage was estimated from the study at 18 °C. The uncertainty contribution therefore describes the possible degradation for 24 months (2 years) at 18°C.

The results of these evaluations are summarised in Table 5.

Table 5: Uncertainties of stability during storage and dispatch. $u_{sts,rel}$ was calculated for a temperature of 60 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of 18 °C and 2 years (24 months).

Element	$u_{sts,rel}$ [%], 60 ºC	$u_{lts,rel}$ [%], 18 ºC
As	1.31	1.43
Cd	1.88	1.08
Cu	2.34	1.39
Hg	0.68	1.66
Pb	2.46	0.91
Se	0.64	3.15
Zn	1.68	1.25

After the certification campaign, the material will be subjected to IRMM's regular stability monitoring programme to further control its stability.

6 Characterisation study

The characterisation of the material was based on an inter-laboratory comparison study. The mass fractions of As, Cd, Cu, Hg, Pb, Se and Zn in ERM-CD200 were determined in different laboratories applying different measurement procedures to demonstrate the absence of measurement bias. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

6.1 Selection of participants

Thirteen laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the field of trace element measurements in algae or similar matrices by submitting results for inter-laboratory comparison exercises or method validation reports. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 was obligatory. Whereas measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (section 2).

Some participants were able to measure one or more elements by two or more independent techniques. Such measurements were listed under separate lab codes as independent measurement results.

6.2 Study setup

The units for this study were selected using a randomly stratified sampling scheme covering the whole batch produced. Each laboratory received a minimum of 2 units (depending on the methods used) of ERM-CD200 and was requested to provide three independent results per

unit. The measurements, including sample preparation (when needed), had to be spread over at least two days to ensure intermediate precision conditions. The water content had to be determined in each unit and results had to be reported on dry mass basis (section 9.5).
Each participant also received a bottle of BCR-279 (Seaweed) as a blind quality control (QC) sample. The results for BCR-279 were used to support the evaluation of the characterisation results.
Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the six results. No approach for the estimation of measurement uncertainty was prescribed, with top-down and bottom-up approaches regarded as equally valid.

6.3 Methods used

A variety of digestion procedures (using HNO_3, H_2O_2 and HF amongst other reagents) with different quantification steps (AAS, ICP-OES and ICP-MS, amongst others) as well as methods without sample preparation (for example, pyrolysis AAS and k_0-NAA) were used to characterise the material. The combination of results from methods based on completely different principles mitigates undetected method bias.

All methods used during the characterisation study are summarised in Annex D. The laboratory code (e.g. L01) is a random number and does not correspond to the order of laboratories in section 2.

6.4 Evaluation of results

The characterisation campaign resulted in between 8 and 14 laboratory means per element. All individual results of the participants, grouped per element are displayed in tabular and graphical form in Annex E.

6.4.1 Technical evaluation

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- appropriate validation of the measurement procedure
- compliance with the analysis protocol: sample preparations and measurements performed on two days, the order of analysis and water content determination.
- absence of values given as below the limit of quantification
- method performance; relative standard deviation (RSD) within and between bottle or day of measurement (≤ 20 %), agreement of the measurement results with the assigned value of the QC sample BCR-279 [12]

Some datasets were rejected as not technically valid, based on the abovementioned criteria. One laboratory measured all samples on one day only, and their results were therefore excluded. A detailed description of the technical evaluation of the data from the remaining labs is given, by element, in Table 6.

Table 6: Datasets that showed non-compliances with the analysis protocol and technical specifications, and action taken

Element	Lab code	Description of problem	Action taken
As	L07	Technical problem due to As concentration higher than method working range	Value not used for evaluation
Cd	L02, L04	Results < LOQ	Values not used for evaluation
Cu	L02 L11	Results < LOQ Difference between days > 20 %	Values not used for evaluation
Hg	L02 L11	Difference between days > 20 % Measurements of QC sample did not agree with assigned values	Values not used for evaluation
Pb	L13	Difference between days > 20 %	Value not used for evaluation
Se	L07, L11, L13 L04	Measurements of QC sample did not agree with assigned values Results < LOQ	Values not used for evaluation
Zn	L02	Measurements of QC sample did not agree with assigned values	Value not used for evaluation

6.4.2 Statistical evaluation

The datasets accepted on technical grounds were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations, (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 7.

Table 7: Statistical evaluation of the technically accepted datasets for ERM-CD200. *p*: number of technically valid datasets

Element	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Average [mg/kg]	*s* [mg/kg]	$s_{between}$ [mg/kg]	s_{within} [mg/kg]
As	14	0	0	yes	55.15	2.46	2.38	1.49
Cd	11	0	0	yes	0.945	0.069	0.069	0.021
Cu	9	1	0	no	1.713	0.098	0.094	0.065
Hg	8	0	0	yes	0.0186	0.0014	0.0013	0.0013
Pb	9	0	0	yes	0.509	0.064	0.062	0.032
Se	8	0	0	yes	0.0882	0.0083	0.0080	0.0060
Zn	13	0	0	yes	25.34	2.10	2.06	1.04

The laboratory means follow normal distributions for most elements and the data does not contain outlying means or variances. The dataset of Cu was not normally distributed because one laboratory mean was found to be an outlier by the statistical tests. However, the outlier agrees with the dataset mean, within the combined uncertainties on the mean and the

measurement. The datasets for all elements are therefore consistent and the means of laboratory means are good estimates of the true value. Standard deviations among laboratories are considerably larger than the standard deviation within laboratories. This shows that confidence intervals of replicate measurements are generally unsuitable as estimates of measurement uncertainty.

In addition to the statistical tests, the agreement of individual laboratories' results with the dataset means was tested according to ERM Application Note 1 [12]. For As, Cd, Cu, Pb and Zn, one or more results did not agree with the dataset within the combined U_{CRM} and reported measurement uncertainty, U_m. The U_m is expected to differ depending on the technique applied. However, large differences were observed in the levels of U_m reported by different labs using similar techniques. Approaches to uncertainty estimation also differ, and it is possible that some labs do not include all sources of uncertainty in their budgets. As a consequence, if a lab's result disagrees with the dataset mean within their reported U_m, it is not possible to conclude that either the result is genuinely different, or the uncertainty was underestimated. To assess the agreement of individual results with dataset means, an attempt was made to estimate realistic U_m by element and technique. However, it was not possible to estimate the expected U_m in cases where only two labs reported results by a similar technique, for a particular element's dataset. Therefore, an arbitrary value of 20 % was considered to represent an acceptable level of U_m for inclusion of a result in the dataset, for each element. Consequently, in testing the agreement of measurement results with the assigned value for the QC sample, and the agreement of results for the samples of ERM-CD200 with the dataset means, U_m of 20 % were used in calculation.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means. Uncertainties are listed by element, before rounding, in Table 8.

Individual results for the characterisation study together with the uncertainty stated by the laboratories are reported in Annex E.

7 Value Assignment

Certified values were assigned, and additional material information is provided in this report.

Certified values are values that fulfil the highest standards of accuracy. Procedures at IRMM require generally pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets in accordance with the Guide to the expression of uncertainty in measurement [3] must be established.

Additional material information refers to values that were obtained in the course of the study. For example, results reported from only one or two laboratories in cases where individual measurement uncertainty is high, would fall under this category.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 8 was assigned as certified value for each element.

u_{char} was estimated as the standard deviation of laboratory means, i.e. $u_{char} = \frac{s}{\sqrt{p}}$ with s and p taken from Table 8.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (section 6), potential between-unit heterogeneity, u_{bb} (section 4) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (section 5). These different contributions were

combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM,\,rel}$) with a coverage factor *k* as

$$U_{CRM,rel} = k \cdot \sqrt{u_{char,rel}^2 + u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2}\,.$$

- u_{char} was estimated as described in section 6.4.2
- u_{bb} was estimated as described in section 4.
- u_{sts} and u_{lts} were estimated as described in section 5.3.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor *k* of 2 was applied, to obtain the expanded uncertainties.
The certified values and their uncertainties are summarised in Table 8.

Table 8: Certified values and their uncertainties for ERM-CD200

Element	Certified value [1] [mg/kg]	$u_{char,\,rel}$ [%]	$u_{bb,\,rel}$ [%]	$u_{sts,\,rel}$ [%]	$u_{lts,\,rel}$ [%]	$U_{CRM,\,rel}$ [%]	U_{CRM} [2] [mg/kg]
As	55	1.19	2.83	1.31	1.43	7.3	4
Cd	0.95	2.20	0.53	1.88	1.08	6.3	0.06
Cu	1.71	1.91	3.74	2.34	1.39	10.0	0.18
Hg	0.0186	2.70	2.55	0.68	1.66	8.3	0.0016
Pb	0.51	4.18	1.99	2.46	0.91	10.6	0.06
Se	0.088	3.34	2.31	0.64	3.15	10.4	0.010
Zn	25.3	2.30	0.92	1.68	1.25	6.5	1.7

[1] reported on dry mass basis (see section 9.5)

[2] expanded (*k* = 2) and rounded uncertainty

7.2 Additional material information

Water content and activity

Water content was determined by Karl-Fischer titration and by an oven drying method, shortly after the filling of the material into glass bottles. The results evaluated as mean values ± standard deviation of separate measurements were 2.8 ± 0.2 g/100 g (n = 10) and 2.4 ± 0.1 g/100 g (n = 6), respectively.

Water activity was determined by using a water activity meter (Aqualab CX3, Decagon, USA) and resulted <0.07 (n = 3).

The water content determined by the laboratories within the characterisation study was in the range of 0.8 % to 4.1 %, and the results within each laboratory were consistent.

Inter-comparison study on particle size analysis (PSA)

Two units of ERM-CD200 were randomly selected from the whole batch produced and measured in duplicate at IRMM (BE) and in triplicate at Sympatec (Clausthal-Zellerfeld, DE). At IRMM, the material was dispersed in 2-propanol and analysed by using a Sympatec Helos F system (IRMM), fitted with a R5 lens. At Sympatec, the material was dispersed in a Quixel system and analysed by mean of a Helos R system using two different instrumental set ups: with a R5 lens system (Sympatec B) and with an extended measurement range where lenses R3 and R5 are combined (Sympatec C). The combination of R5 and R3 lenses was found to allow the easier identification of differences in < 10 µm particles. The optical

concentration of the dispersed powder was within 15-25 %. The results of this study are summarised in Table 9.

Table 9: Cumulative particle size distribution, expressed as average particle size (µm) ± *s* (µm) of 4 and 18 replicate measurements for IRMM, Sympatec B and C, respectively.

	Size class / %				
	X_{10}	X_{16}	X_{50}	X_{84}	X_{90}
IRMM	10.86 ± 0.61	16.74 ± 0.69	56.42 ± 1.83	112.59 ± 2.53	128.26 ± 3.21
Sympatec B	8.77 ± 0.14	14.59 ± 0.26	53.75 ± 0.79	108.59 ± 1.10	123.81 ± 0.83
Sympatec C	8.01 ± 0.15	13.60 ± 0.22	50.98 ± 0.92	105.45 ± 2.02	121.89 ± 2.29

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

The measurands are clearly defined total element mass fractions. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias. The measurands are therefore structurally defined and independent of the measurement method.

Quantity value

Only validated methods were used for the determination of the assigned values. Different calibration standards of known purity and specified traceability of their assigned values were used and all relevant input parameters were calibrated. The individual results are therefore traceable to the SI. This is also confirmed by the agreement among the technically accepted datasets. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are also traceable to the SI.

8.2 Commutability

Many measurement procedures include one or more steps, which select specific (or specific groups of) analytes from the sample for the subsequent steps of the measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all the analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions expressing this concept. For instance, the CSLI Guideline C-53A [11] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and, thus, is a crucial characteristic in case of the application of different measurement methods. When commutability of a CRM is not established in such cases, the results from routinely used methods cannot be legitimately

compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

ERM-CD200 is a natural seaweed material. The analytical behaviour will be the same as for a routine sample of dried seaweed. For samples other than seaweed, the commutability has to be assessed.

9 Instructions for use

9.1 Storage conditions

The material shall be stored at 18 °C ± 5 °C in the dark. Care shall be taken to avoid change of the moisture content once the vial is open, as the material is hygroscopic. Storage in a desiccator is recommended.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened vials.

9.2 Safety and protection for the environment

The usual laboratory safety measures apply.

9.3 Preparation and use of the material

The vial shall be shaken by turning upside down for at least 2 minutes before opening to ensure re-homogenisation of the material.

9.4 Minimum sample intake

The minimum sample intake is 200 mg for all elements.

9.5 Dry mass correction

Dry mass determination shall be carried out on a separate portion of at least 0.4 g of material by drying in an oven at 105 °C ± 2 °C until constant mass (separate weighing should not differ by more than 0.2 mg). Weighing of the samples for dry mass determination and weighing for the analysis shall be done at the same time to avoid differences due to possible take up of moisture by the material.

9.6 Use of the certified value

The main purpose of the material is to assess method performance, i.e. for checking accuracy of analytical results/calibration. As any reference material, it can also be used for control charts or validation studies.

Comparing an analytical result with the certified value

A result is unbiased if the combined uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, www.erm-crm.org [12].

For assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_m) with the uncertainty of the certified value (u_{CRM}): $u_\Delta = \sqrt{u_m^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_m \leq U_\Delta$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95 %.

Use as a calibrant

It is not recommended to use ERM-CD200 as calibrant. If used nevertheless, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty.

Use in quality control charts

The material ERM-CD200 can be used for quality control charts. Different CRM-units will give the same result as heterogeneity was included in the uncertainties of the certified values.

10 Acknowledgments

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The authors would also like to acknowledge Sympatec Benelux for the participation in the PSA proficiency testing.

11 References

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Annexes

Annex A: Results of the homogeneity measurements

Annex B: Results of the short-term stability measurements

Annex C: Results of the long-term stability measurements

Annex D: Summary of methods used in the characterisation study

Annex E: Results of the characterisation measurements

CERTIFICATION REPORT: The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (*Fucus vesiculosus*): ERM-CD200

Annex A: Results of the homogeneity measurements

Graphs present mass fractions of bottle means relative to the grand mean, against bottle number, and individual measurement replicates, against sequence number. Vertical bars are a confidence interval of 95 % derived from s_{wb} of the homogeneity study.

Figure A1: As

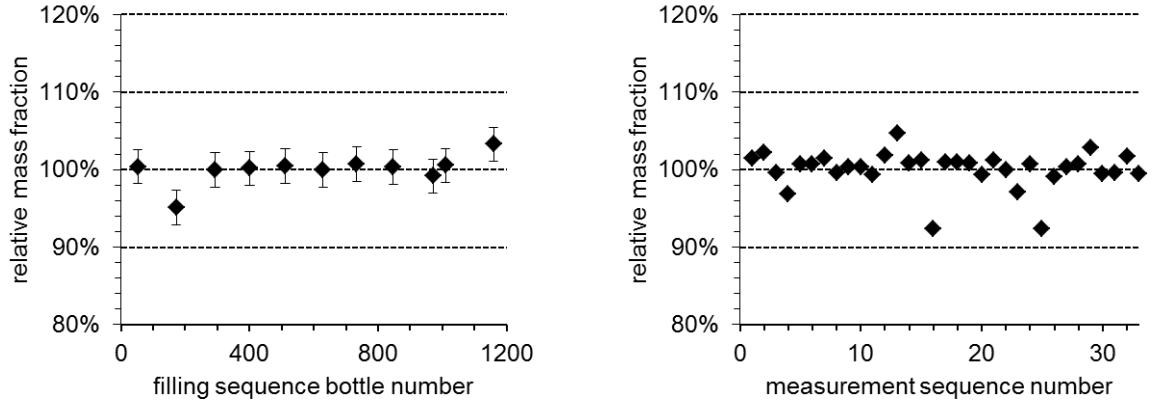


Figure A2: Cd

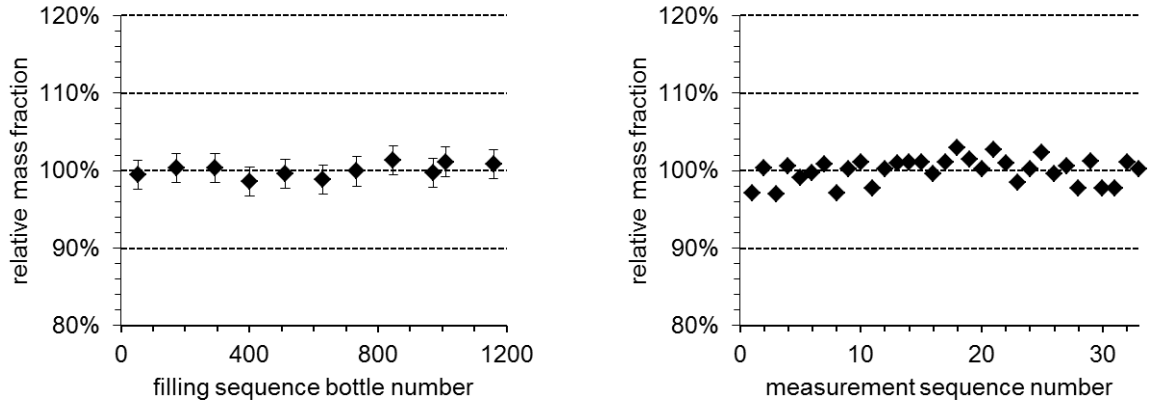


Figure A3: Cu

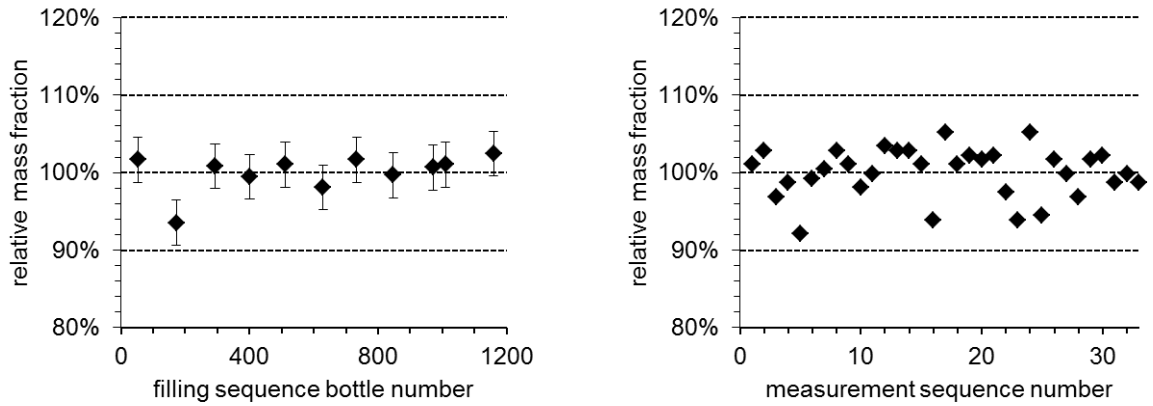


Figure A4: Hg

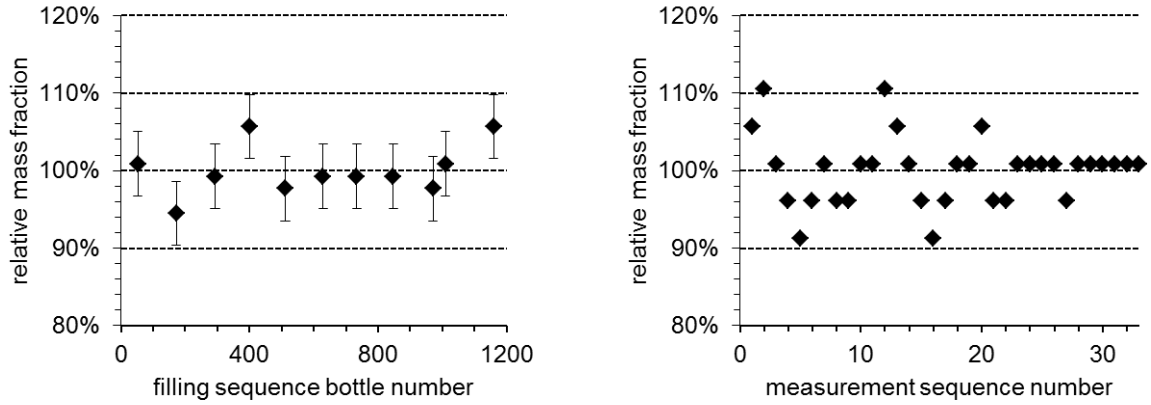


Figure A5: Pb

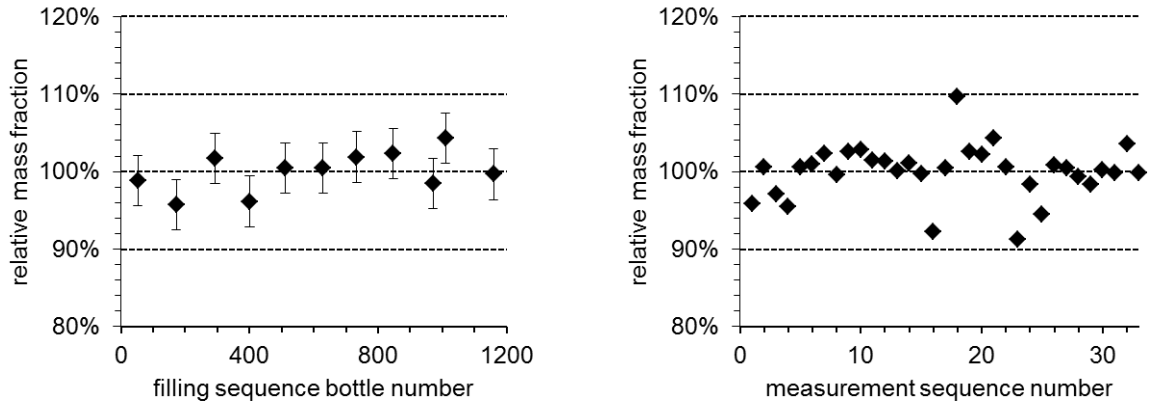


Figure A6: Se

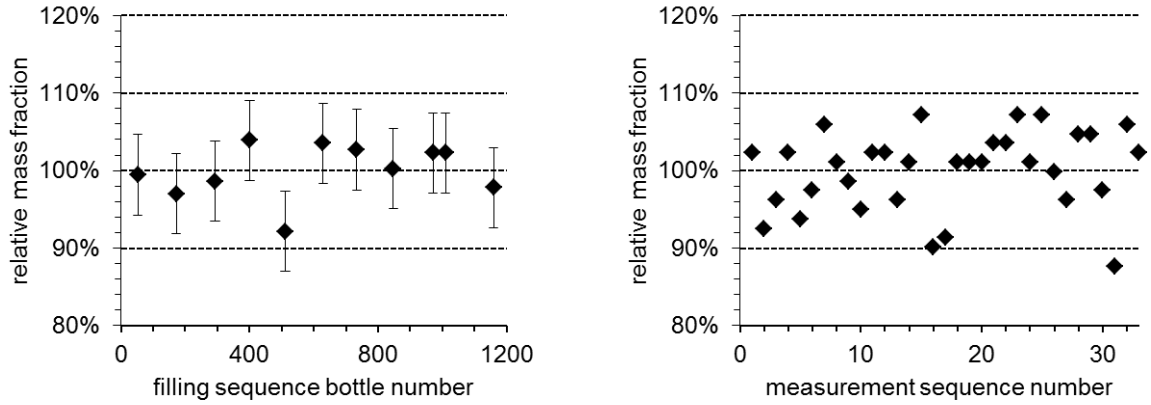
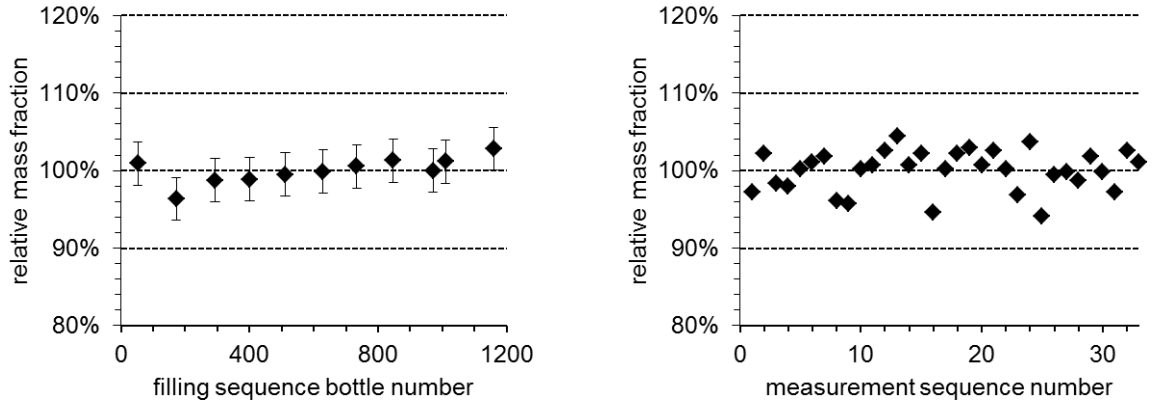


Figure A7: Zn


120%
110%
100%
90%
80%
relative mass fraction
0
400
800
1200
filling sequence bottle number
120%
110%
100%
90%
80%
relative mass fraction
0
10
20
30
measurement sequence number

CERTIFICATION REPORT: The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (*Fucus vesiculosus*): ERM-CD200

Annex B: Results of the short-term stability measurements

Graphs present the mean mass fractions measured at each time-point relative to the mean at time zero, against the time that the samples were held at 18 ºC or 60 ºC. Vertical bars represent the 95 % confidence interval of the measurements, based on the variance of measurements for each time-point calculated by ANOVA. Dotted lines represent the uncertainty associated to short-term stability, u_{STS}.

Figure B1: As

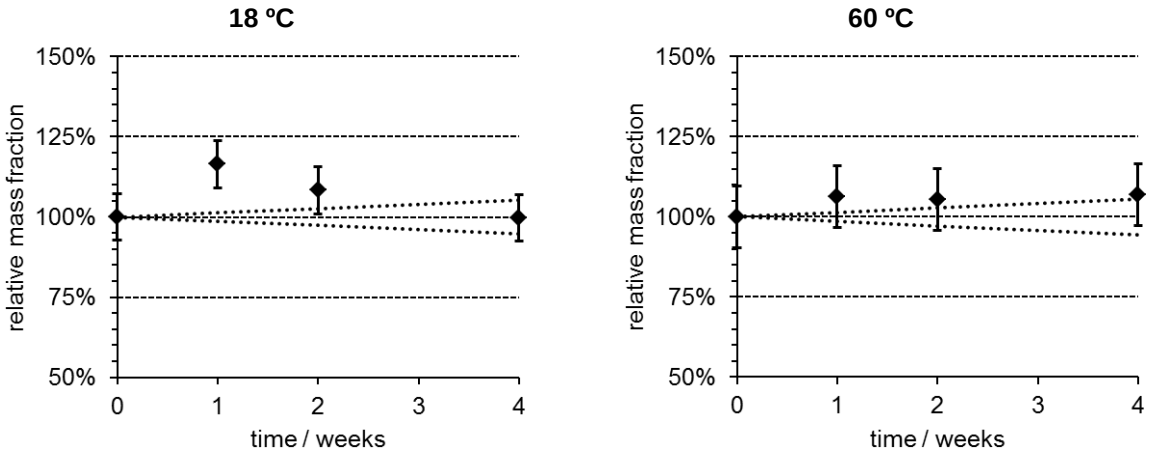


Figure B2: Cd

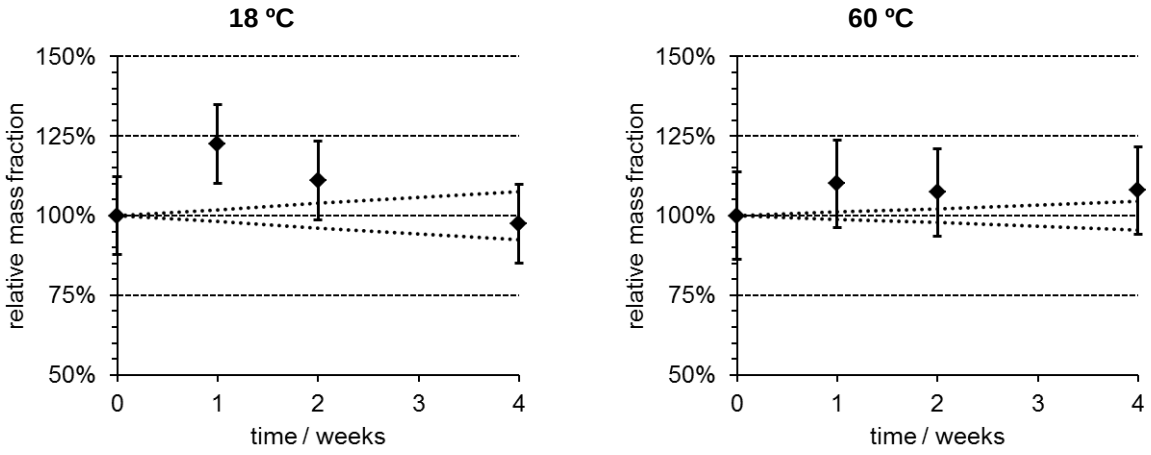


Figure B3: Cu

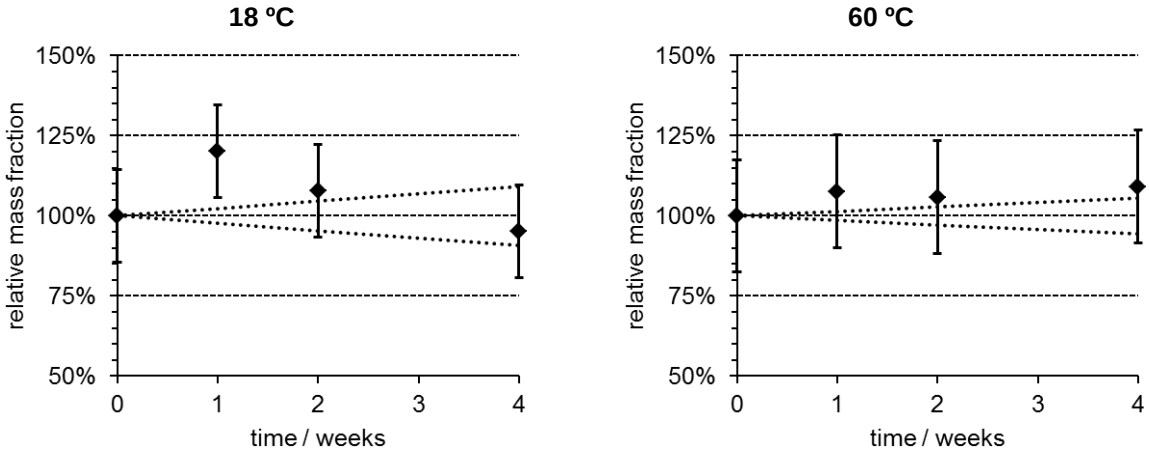


Figure B4: Hg

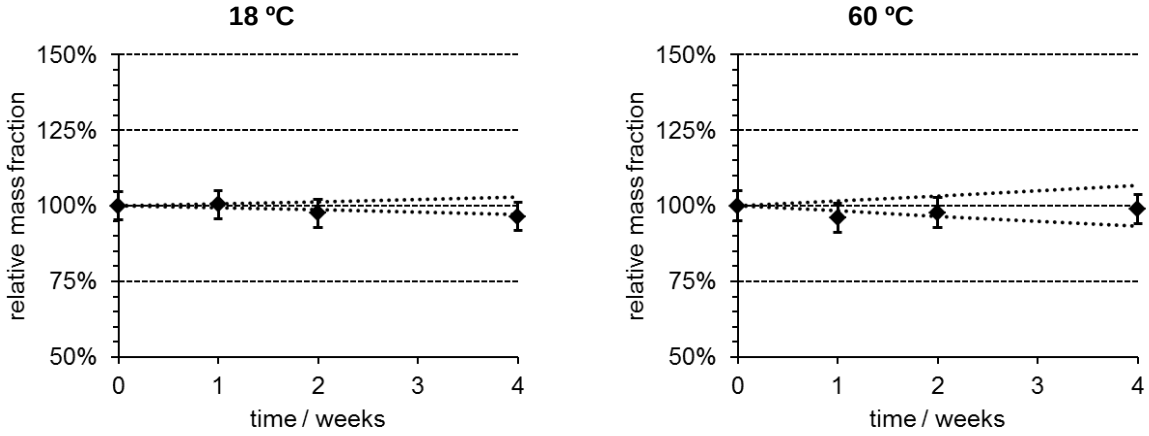


Figure B5: Pb

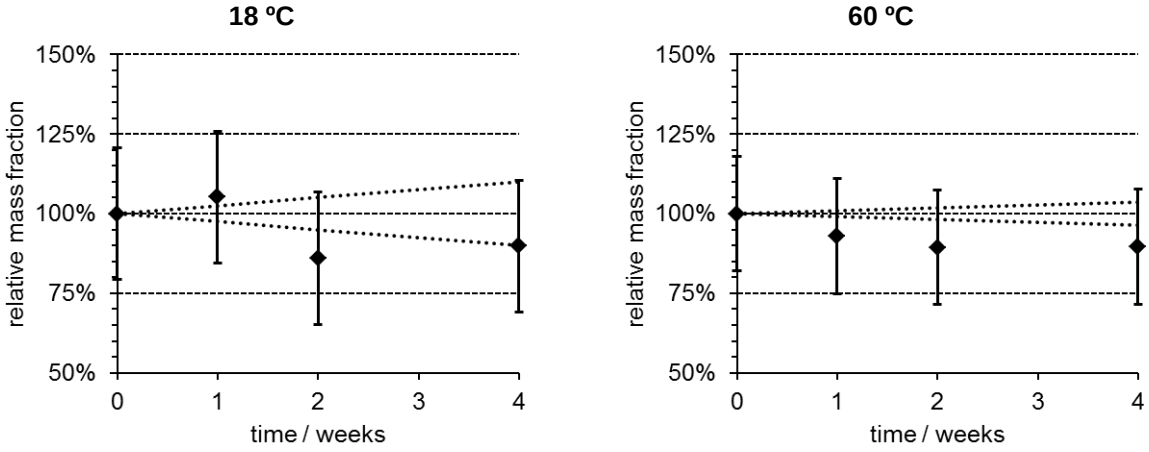


Figure B6: Se

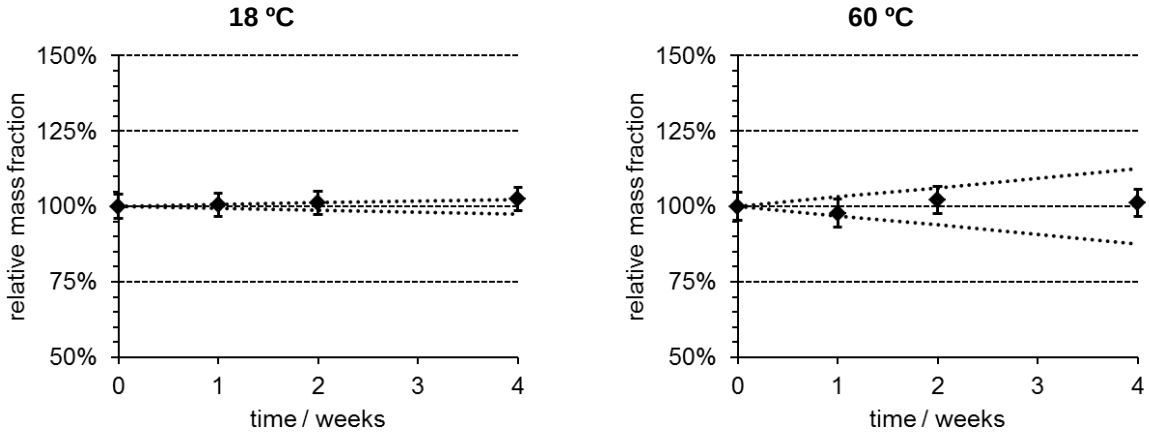
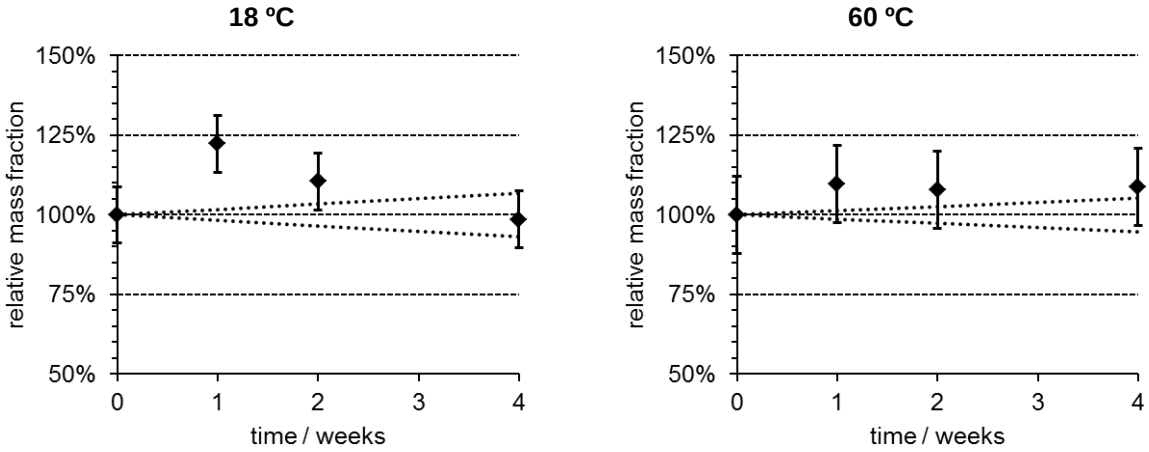


Figure B7: Zn



CERTIFICATION REPORT: The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (*Fucus vesiculosus*): ERM-CD200

Annex C: Results of the long-term stability measurements

Graphs present the mean mass fractions measured at each time-point relative to the mean at time zero, against the time that the samples were held at 18 ºC. Vertical bars represent the 95 % confidence interval of the measurements, based on the variance of measurements for each time-point calculated by ANOVA. Dotted lines represent the uncertainty associated to long-term stability, u_{LTS}.

Figure C1: As

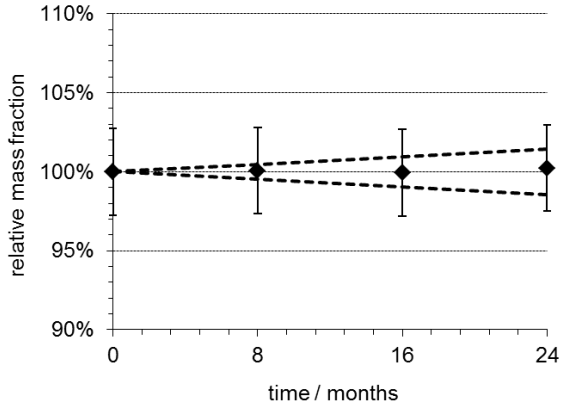


Figure C2: Cd

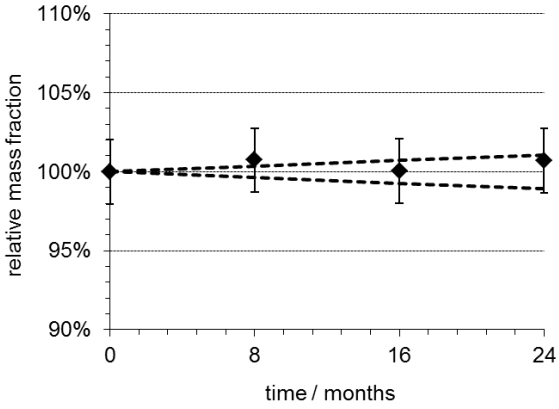


Figure C3: Cu

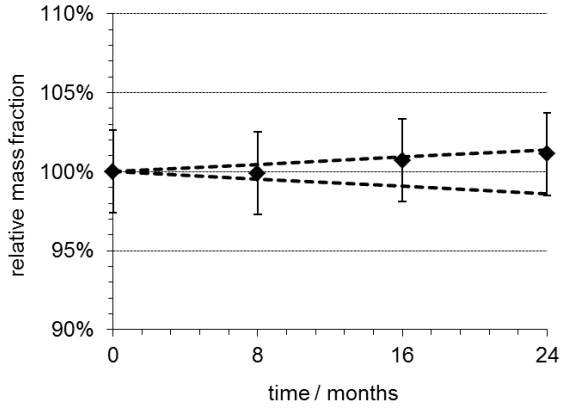


Figure C4: Hg

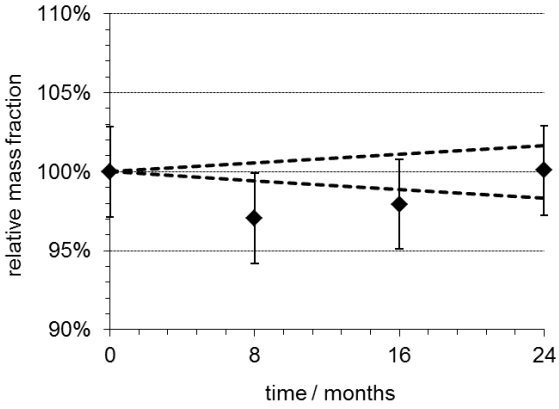


Figure C5: Pb

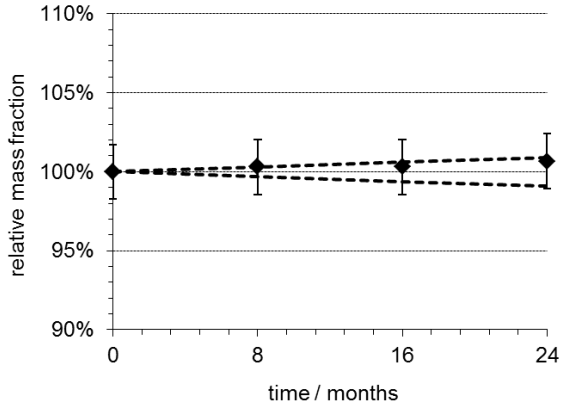


Figure C6: Se

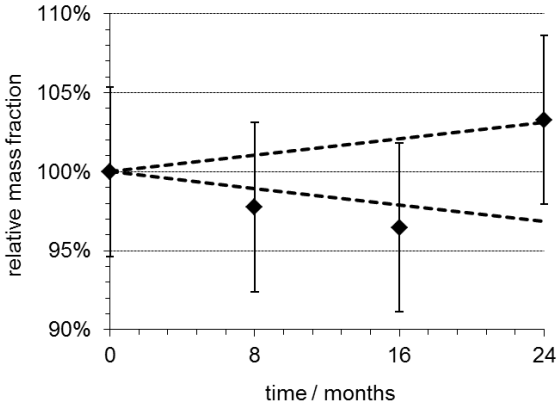
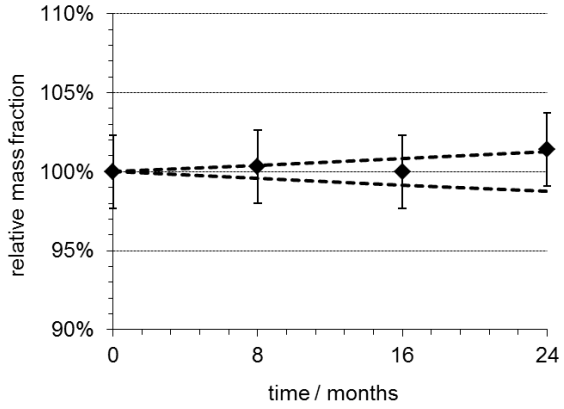


Figure C7: Zn



CERTIFICATION REPORT

The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (Fucus vesiculosus): ERM- CD200

Annex D: Summary of methods used in the characterisation

Note that measurement methods are given as reported by the laboratories, and may not follow the terminology of the Guide to the Expression of Uncertainty in Measurements, [ISO/IEC Guide 98-3:2008], or the International Vocabulary of Metrology – Basic and General Concepts and Associated Terms, [ISO/IEC Guide 99:2007].

Table D1: Summary of the methods used for the homogeneity study

Parameter (measurement method)	Sample mass (g)	Sample preparation	Calibration	Instrumentation
As, Cd, Cu,Hg, Pb, Se, Zn (ICP-SFMS)	0.5	Closed MW –assisted digestion (CEM-MARS5) with 5 mL HNO_3 + 0.1mL HF. Dilution with 10% HNO_3	2-points external calibration after internal correction, with Ultra Scientific (USA) standards	Thermo Electron Corp. Cetac

Table D2: Summary of the methods used for the short term stability study

Parameter (measurement method)	Sample mass (g)	Sample preparation	Calibration	Instrumentation
As, Cd, Cu, Pb, Zn (ICP-OES)	1	Method EPA3052	Minimum 5-points external calibration, with Ultra Scientific (USA) standards	Varian Vista-MPX
Hg (CV-AAS) Se (ET-AAS)	0.5	Closed MW –assisted digestion (Paar Multiwave 3000) with 4mL HNO_3 + 2mL H_2O_2 + 0.5mL HF	Minimum 6-points external calibration, with Ultra Scientific (USA) standards	Perkin Elmer FIMS 400 (CV-AAS); Perkin Elmer FIMS 400 coupled to Perkin Elmer AA800 (ET-AAS)

Table D3: Summary of the methods used for the long term stability study

Parameter (measurement method)	Sample mass (g)	Sample preparation	Calibration	Instrumentation
As, Cd, Cu,Hg, Pb, Se, Zn (ICP-MS)	0.5	Closed MW –assisted digestion with 8mL HNO_3 + 2mL H_2O_2	6-points external calibration, with NIST (USA) high purity standards	Agilent 7500ce

Table D4: Summary of the methods used for the characterisation study

Lab/Method code	Parameter (measurement method)	Sample mass (g)	Sample preparation	Calibration	Instrumentation
L00	As, Zn (k_0-NAA)	0.4	n.a.	IRMM-530R	k_0-NAA
L01	As, Cd, Cu, Hg, Pb, Se, Zn (ICP-SFMS)	0.3	Closed MW –assisted digestion (Milestone-MARS5) with 5 mL HNO_3 + 0.05 mL HF. Dilution with 10% HNO_3	2-points external calibration after internal correction, with Ultra Scientific (USA) standards	Thermo Scientific Element 2
L02	As, Cd, Cu, Hg, Se, Zn (INAA)	0.2	n.a.	Calibration of the HPGe detector made by using calibrated gamma-ray sources	HPGe detectors (Ortec)
L03	As, Cd, Hg, Pb (ICP-MS)	0.5	Closed MW –assisted digestion (Multiwave 3000) with5 mL HNO_3. Dilution with milli-Q water	Standard addition by using SCP Science standards	Agilent 7500ce
L04	As, Cd, Se, Zn (k_0-NAA)	0.25	n.a.	IRMM-530R	TRIGA Mark II reactor (GA), HPGe detector 40% (Ortec)
L05	Hg (DMA)	0.03	Thermal combustion integrated at 650 °C	10-points external calibration by using JSI standards	Milestone DMA
L06	As, Se, Zn (INAA)	0.2	n.a.	Calibration with Inorganic Ventures standards	Triga Mark II Nuclear Reactor
L07	As, Cd, Cu, Pb, Zn (ICP-MS) Se (ETAAS)	0.5 (As, Cd, Cu, Pb, Zn) 0.1 (Se)	Open digestion block with 10 mL HNO_3 for As, Cd, Cu, Pb, Zn. Digestion with a mixture of HNO_3, H_2SO_4 and $HClO_4$ in 5 mL organic solvent. Dilution with milli-Q water	3-points external calibration by using AccuStandard Inc. standards	Perkin Elmer Elan 6000 (ICP-MS) Varian AA280Z (ETAAS)
L08	As, Cd, Cu, Hg, Pb, Se, Zn (ICP-SFMS)	0.2	Closed MW –assisted digestion (CEM-MARS Express) with 2 mL HNO_3 + 0.5 mL H_2O_2 and 0.5 mL HF. Dilution with milli-Q water	6-points external calibration by using NIST standards	Thermo Scientific Element 2
L09	Cd, Cu, Pb, Se and Zn (ID-ICP-MS) Hg (DMA)	0.8 (Cd, Cu, Pb, Se, Zn) 0.2 (Hg)	High pressure asher (ANTON PAAR HPA-S) with 4 mL HNO_3 + 0.5 mL H_2O_2. Solutions were spiked with isotopic enriched spike solutions for the different metals.	Calibration by using Merck, Spex or Alfa Aesar standards	Thermo Scientific (ID-ICP-MS) Milestone DMA-80 (DMA)

Lab/Method code	Parameter (measurement method)	Sample mass (g)	Sample preparation	Calibration	Instrumentation
L10	As, Cd, Cu, Pb, Se and Zn (ICP-MS)	1	High pressure asher (ANTON PAAR HPA-S) with 4 mL HNO_3 + 0.5 mL H_2O_2.	5-points external calibration by using Spex standards	Thermo Scientific (ICP-MS)
L11	As, Cd, Cu, Pb, Se and Zn (ICP-MS)	0.5	Microwave digestion using nitric acid.	External Calibration (range 0.5-100ug/L), using linear regression line. Sample conc = (soln conc-blk conc)*dilution/weight, Stock mixed elements 1000mg/L calibration solution was purchased from SPEX Certiprep, Reference Material Producer CERT # 2495.01	ICP-MS
L12	As, Cd, Cu, Pb, Se and Zn (ICP-MS)	0.25	0.25 g digested in 0.4 ml HCl + 1.6 ml HNO_3 using PTFE vessels in a pressure vessel, microwave heating	External calibration, (seven calibrants spanning three orders of magnitude), VWR (BDH, Prolabo) standard (Traceable to NIST)	Agilent 7700x ICP-MS [helium mode]
L13	As, Cd, Cu, Pb (GFAAS)	0.2	Sample is digested in microwave (high pressure and temperature), with concentrated nitric acid and hydrogen peroxide. After digestion, sample is diluted to 10ml with water. Measurements made with 2 matrix modifiers: Pd (1000mg/l) and Magnesium nitrate (1000 mg/l) for As, Cu and DHPA (1%l) and Magnesium nitrate (1000 mg/l) for Cd, Pb	external calibration curves with 4 standards, Arsenic (1000 mg/l): Fluka 11082, Cadmium (1000 mg/l): Fluka 20895, Copper (1000 mg/l): Fluka 61147, Lead (1000 mg/l): Fluka 16595	Graphite furnace
L13	Se (HGAAS)	0.2	Sample is digested in microwave (high pressure and temperature), with concentrated nitric acid and hydrogen peroxide. After digestion, sample is transferred to muffle (1h, 450ºC) with Magnesium nitrate solution. After ashes are white, they are transferred and diluted to 10ml with diluted HCl.	external calibration curve with 4 standards (1 to 10 ug/l), Selenium (1000 mg/l): Fluka 84896	Hydride generation Wavelength: 196,0nm

Lab/Method code	Parameter (measurement method)	Sample mass (g)	Sample preparation	Calibration	Instrumentation
L13	Zn (FAAS)	0.2	Sample is digested in microwave (high pressure and temperature), with concentrated nitric acid and hydrogen peroxide. After digestion, sample is diluted to 10ml with 2% lithium solution	external calibration curve with 4 standards (0,1 to 2,5 mg/l), Zinc (1000 mg/l): Fluka 6457	Flame atomic absorption. Wavelength: 213,9nm
L14	As, Cd, Cu, Hg, Se (AAS)	0.5	Closed microwave digestion (Multiwave 3000); 4 mL HNO_3 (68% subboiled) + 2 mL H_2O_2 (30% p.a.) + 0.5 mL HF (40% suprapur)	external calibration with Baker Instra Analyzed ICP Standards (traceable to NIST SRMs)	As, Cd and Cu by GFAAS, Hg by CVAAS and Se by HGAAS
L15	As, Cd, Cu, Pb and Zn (ICP-MS)	0.5	Closed microwave digestion (Multiwave 3000); 4 mL HNO_3 (68% subboiled) + 2 mL H_2O_2 (30% p.a.) + 0.5 mL HF (40% suprapur)	external calibration with Baker Instra Analyzed ICP Standards (traceable to NIST SRMs)	ICP-MS
L16	As, Zn (ICP-OES)	0.5	Closed microwave digestion (Multiwave 3000); 4 mL HNO_3 (68% subboiled) + 2 mL H_2O_2 (30% p.a.) + 0.5 mL HF (40% suprapur)	external calibration with Baker Instra Analyzed ICP Standards (traceable to NIST SRMs)	ICP-OES

CERTIFICATION REPORT

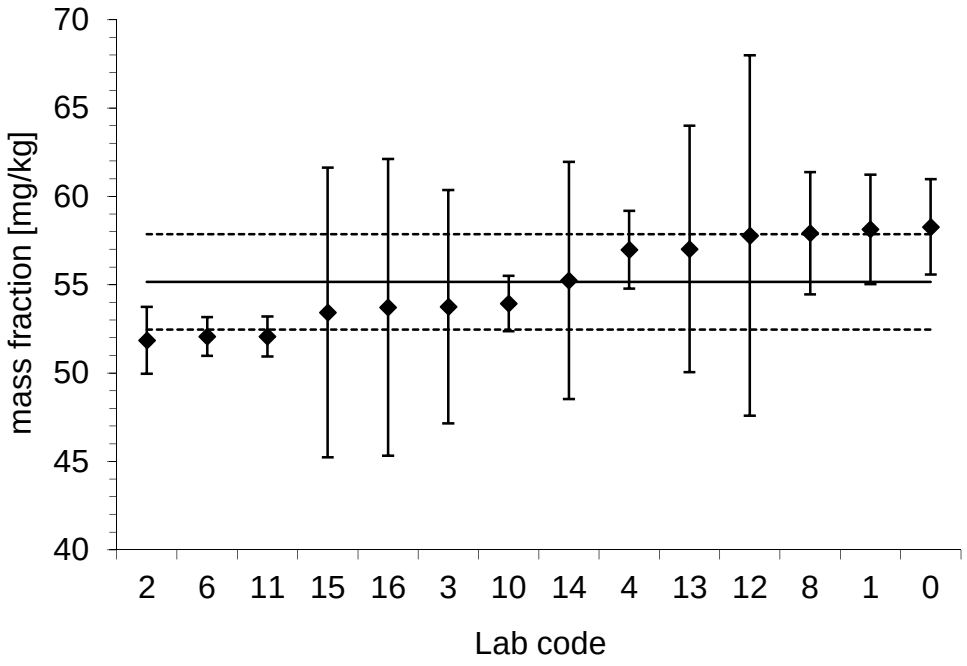
The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (*Fucus vesiculosus*): ERM-CD200

Annex E: Results of the characterisation measurements

Tables present the results of characterisation measurements (corrected to dry mass) and the estimated uncertainties of the measurements, as reported by participants. Some reported values were rounded for formatting reasons. Graphs show expanded uncertainties as stated by the laboratories, the certified value indicated by a solid line, and the expanded uncertainty on the certified value indicated by dotted lines.

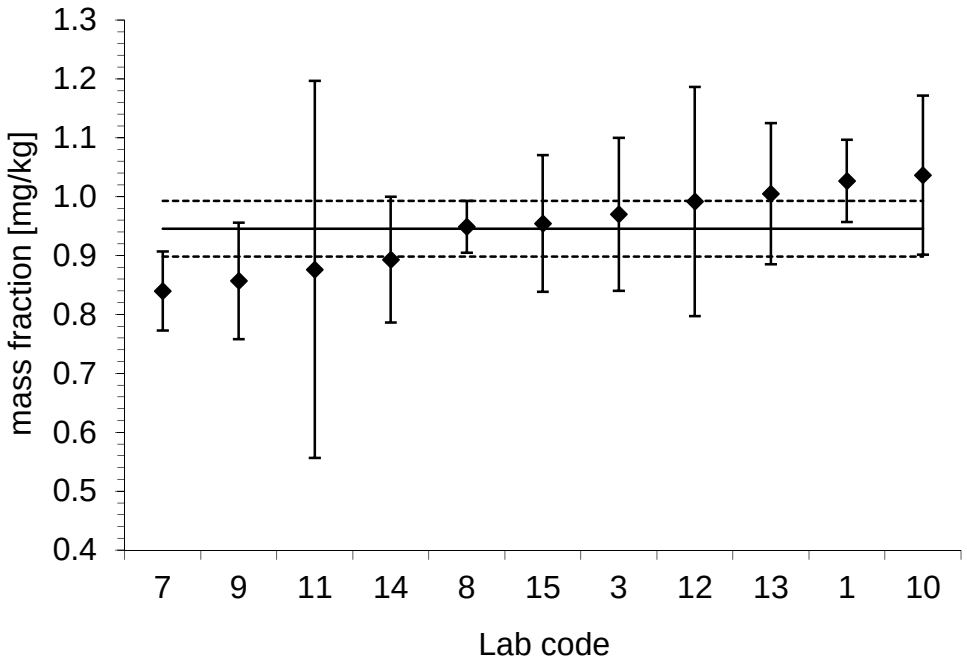
As

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k_0-NAA	58	57.7	57.5	59.6	58.4	58.4	58.267	2.7
1	ICP-SFMS	57	58.8	60.3	57	57.6	58.1	58.133	3.1
2	INAA	52.9	51.8	52.1	51.6	51.8	50.9	51.85	1.9
3	ICP-MS	54.95	55.13	55.53	52.69	52.35	51.9	53.758	6.6
4	k_0-INAA	57.8	56.4	56.2	58.1	56.6	56.8	56.983	2.2
6	INAA	52.9	52.46	51.62	52.5	51.11	51.8	52.065	1.1
8	ICP-SFMS	56.894	58.052	57.765	58.103	59.586	57.113	57.919	3.46
10	ICP-SFMS	53.796	54.041	53.701	53.929	53.323	54.811	53.933	1.567
11	ICP-MS	51.9	52.7	57.6	50.7	50.1	49.4	52.067	1.128
12	ICP-MS	59.85	59.36	59.98	52.45	56.8	58.26	57.783	10.2
13	GFAAS	59.15	56.78	58.18	57.53	54.96	55.54	57.023	6.98
14	GFAAS	55.9	54.1	53.8	56.2	55.6	55.8	55.233	6.71
15	ICP-MS	54.7	53	54.1	53.5	53.5	51.8	53.433	8.2
16	ICP-OES	53.8	53.7	51.5	55.9	53.2	54.2	53.717	8.39
Results not included in the characterisation dataset									
7	ICP-MS	38.6	41.4	36	36.6	40.4	41.3	39.05	3.124



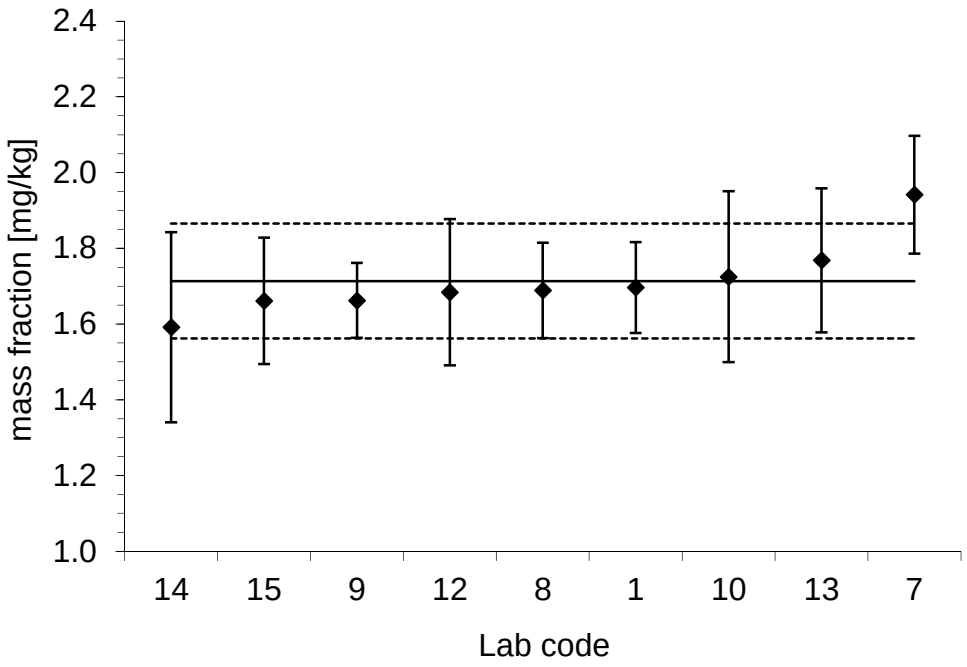
Cd

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	1.04	1.02	1.03	1.03	1.02	1.02	1.0267	0.07
3	ICP-MS	0.96	0.95	0.97	1	0.98	0.96	0.9700	0.13
7	ICP-MS	0.881	0.856	0.828	0.836	0.825	0.813	0.8398	0.0672
8	ICP-SFMS	0.941	0.958	0.935	0.965	0.94	0.955	0.9490	0.044
9	ID- ICP-SFMS	0.8702	0.9252	0.8171	0.8283	0.8502	0.8511	0.8570	0.0991
10	ICP-SFMS	1.0266	1.0259	1.0467	1.0533	1.0399	1.0267	1.0365	0.135
11	ICP-MS	0.842	0.857	0.919	0.879	0.914	0.847	0.8763	0.32
12	ICP-MS	1.0057	0.9864	0.9764	0.9705	0.9883	1.0233	0.9918	0.1944
13	GFAAS	1.04	1.01	1.02	1.01	0.97	0.98	1.005	0.12
14	GFAAS	0.892	0.894	0.883	0.902	0.895	0.892	0.8930	0.107
15	ICP-MS	0.958	0.966	0.959	0.952	0.941	0.95	0.9543	0.116
Results not included in the characterisation dataset									
2	INAA	< 12	< 12	< 11	< 13	< 11	< 11		
4	k_0-INAA	< 1.3	< 1.1	< 1.0	< 1.2	< 1.3	< 1.1		



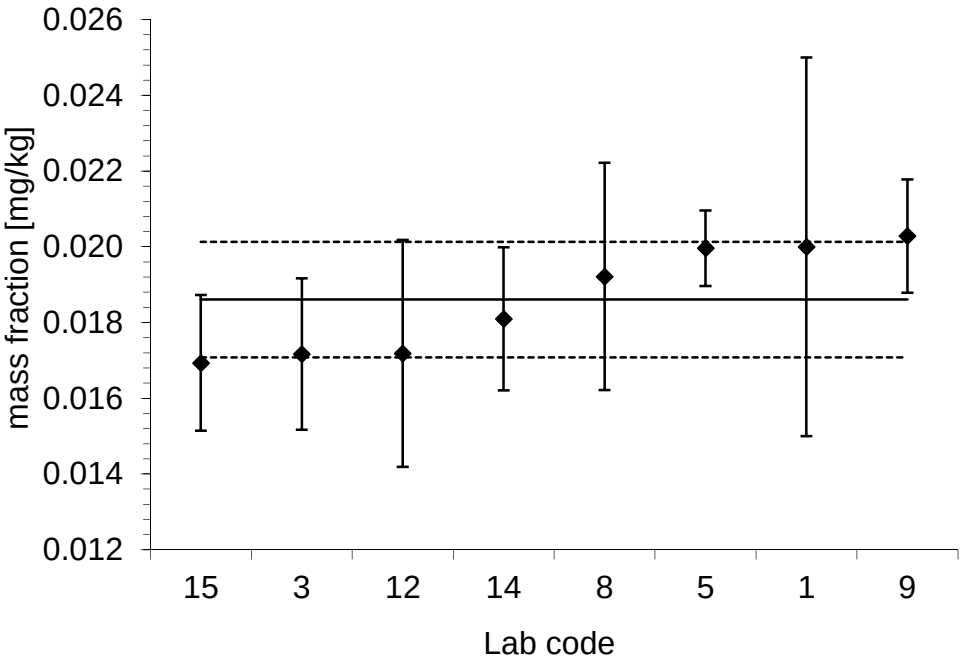
Cu

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	1.69	1.65	1.76	1.67	1.71	1.7	1.6967	0.12
7	ICP-MS	2.01	2.16	1.84	1.81	1.98	1.85	1.9417	0.1553
8	ICP-SFMS	1.713	1.682	1.643	1.744	1.651	1.701	1.689	0.126
9	ID- ICP-SFMS	1.6750	1.6650	1.6841	1.5933	1.6744	1.6832	1.6625	0.0991
10	ICP-SFMS	1.6737	1.7110	1.7537	1.6484	1.7994	1.7641	1.7250	0.226
12	ICP-MS	1.688	1.665	1.652	1.628	1.718	1.755	1.6843	0.193
13	GFAAS	1.69	1.69	1.71	1.8	1.86	1.86	1.7683	0.19
14	GFAAS	1.65	1.54	1.55	1.58	1.56	1.67	1.5917	0.251
15	ICP-MS	1.67	1.67	1.71	1.65	1.61	1.66	1.6617	0.167
Results not included in the characterisation dataset									
2	INAA	< 120	< 120	< 120	< 120	< 120	< 120		
11	ICP-MS	1.45	1.48	1.65	1.89	2	1.9	1.7283	2.626



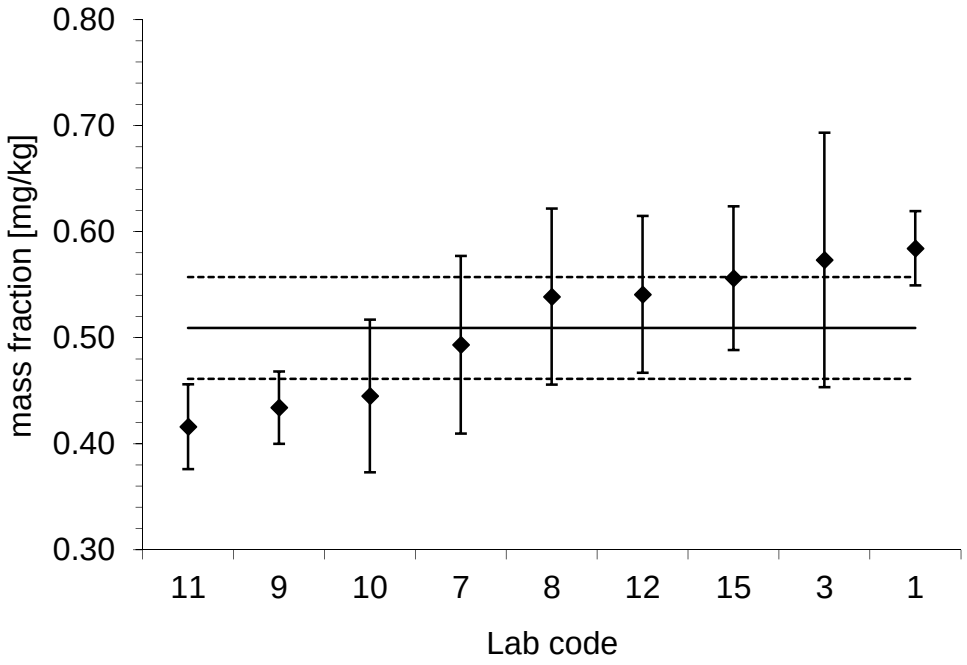
Hg

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.018	0.022	0.02	0.019	0.02	0.021	0.0200	0.005
3	ICP-MS	0.016	0.018	0.016	0.018	0.018	0.017	0.0172	0.002
5	DMA	0.0183	0.0201	0.0204	0.0195	0.0222	0.0193	0.0200	0.001
8	ICP-SFMS	0.017	0.0173	0.0197	0.0221	0.0197	0.0195	0.0192	0.003
9	ID- ICP-SFMS	0.0208	0.0208	0.0209	0.0193	0.0201	0.0198	0.0203	0.0015
12	ICP-MS	0.0146	0.0153	0.0201	0.0188	0.0178	0.0165	0.0172	0.003
14	CVAAS	0.0181	0.0189	0.0179	0.0181	0.018	0.0176	0.0181	0.0019
15	CVAFS	0.0174	0.0174	0.0179	0.0169	0.0167	0.0153	0.0169	0.0018
Results not included in the characterisation dataset									
2	INAA	0.0226	0.0251	0.0235	0.0189	0.0149	0.017	0.0203	0.002
11	ICP-MS	0.04	0.037	0.036	0.036	0.038	0.031	0.0363	0.067



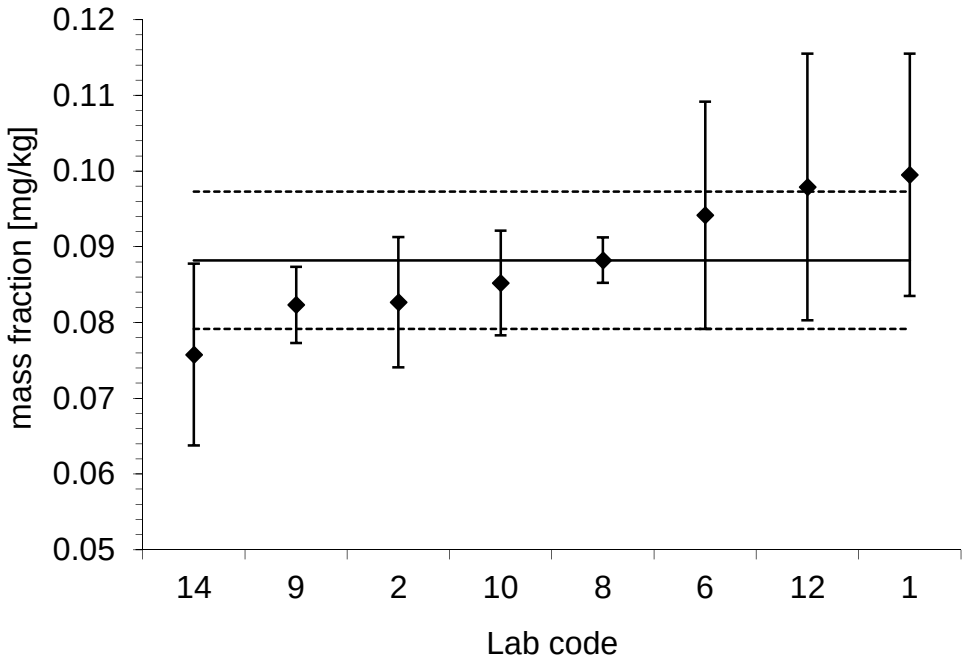
Pb

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.585	0.59	0.584	0.592	0.582	0.573	0.5843	0.035
3	ICP-MS	0.59	0.49	0.52	0.58	0.6	0.66	0.5733	0.12
7	ICP-MS	0.492	0.5	0.482	0.501	0.497	0.488	0.4933	0.0839
8	ICP-SFMS	0.53	0.575	0.528	0.517	0.523	0.559	0.5387	0.083
9	ID- ICP-SFMS	0.4328	0.4561	0.4251	0.4134	0.4297	0.4476	0.4341	0.0341
10	ICP-SFMS	0.4673	0.4115	0.4390	0.4370	0.4814	0.4338	0.4450	0.072
11	ICP-MS	0.357	0.374	0.383	0.476	0.497	0.409	0.416	0.04
12	ICP-MS	0.5271	0.5191	0.5288	0.5694	0.5644	0.5363	0.5409	0.074
15	ICP-MS	0.556	0.56	0.564	0.558	0.539	0.56	0.5562	0.0677
Results not included in the characterisation dataset									
13	GFAAS	0.347	0.357	0.378	0.295	0.305	0.275	0.3262	0.045



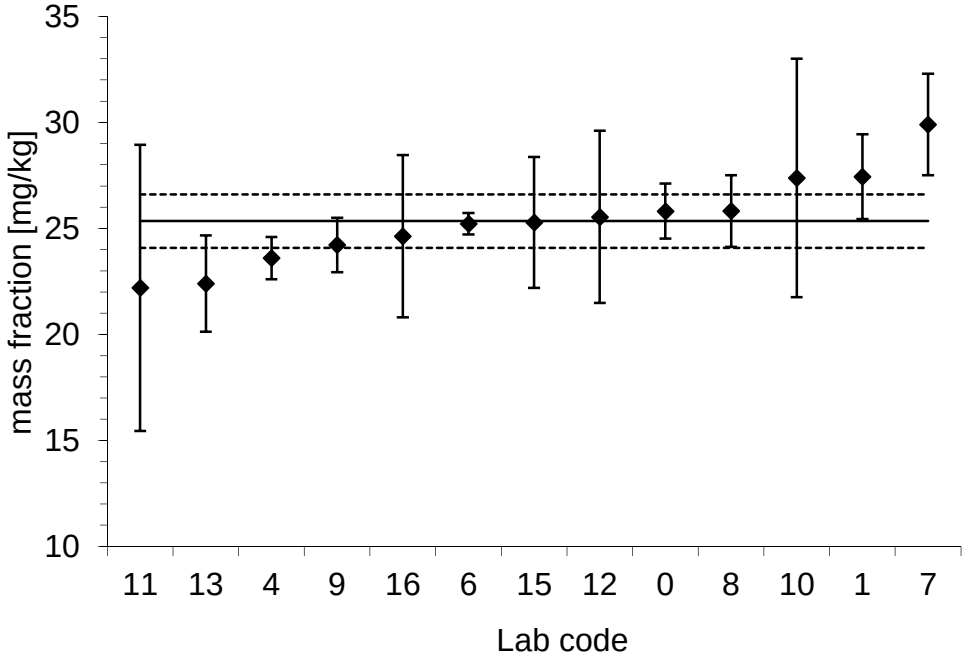
Se

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
1	ICP-SFMS	0.098	0.103	0.099	0.098	0.105	0.094	0.09950	0.016
2	INAA	0.076	0.09	0.075	0.088	0.082	0.085	0.08267	0.0086
6	INAA	0.089	0.096	0.084	0.098	0.105	0.093	0.09417	0.015
8	ICP-SFMS	0.0869	0.0885	0.0859	0.0876	0.0905	0.09	0.08823	0.003
9	ID- ICP-SFMS	0.07997	0.08349	0.08485	0.07913	0.08252	0.08399	0.08233	0.00504
10	ICP-SFMS	0.08330	0.08539	0.08346	0.08416	0.08755	0.08742	0.08521	0.0069
12	ICP-MS	0.1007	0.0865	0.0802	0.1074	0.1027	0.1099	0.09790	0.0176
14	HG-AAS	0.0779	0.0771	0.0797	0.0661	0.0767	0.0771	0.07577	0.012
Results not included in the characterisation dataset									
4	k_0-INAA	< 0.10	< 0.09	< 0.09	< 0.08	< 0.11	< 0.09		
7	ICP-MS	0.068	0.074	0.075	0.062	0.061	0.06	0.06667	0.01
11	ICP-MS	0.394	0.561	0.353	0.323	0.284	0.346	0.37683	0.6
13	HG-AAS	0.061	0.062	0.059	0.07	0.073	0.078	0.06717	0.012



Zn

Lab code	Technique	replicate 1 [mg/kg]	replicate 2 [mg/kg]	replicate 3 [mg/kg]	replicate 4 [mg/kg]	replicate 5 [mg/kg]	replicate 6 [mg/kg]	mean [mg/kg]	Expanded uncertainty [mg/kg]
0	k_0-NAA	25.5	26.4	25.9	25.5	26	25.6	25.82	1.3
1	ICP-SFMS	27.9	27.9	27.5	27.4	27.2	26.7	27.43	2
4	k_0-INAA	23	23.1	23.3	24.6	23.5	24.1	23.60	1
6	INAA	25.1	25.44	24.87	25.59	25.31	25.02	25.22	0.5
7	ICP-MS	29.3	30.7	28.4	28.9	30	32.1	29.90	2.392
8	ICP-SFMS	26.194	25.741	25.261	26.511	25.171	26.026	25.82	1.69
9	ID- ICP-SFMS	23.003	23.658	24.290	25.240	24.902	24.226	24.22	1.287
10	ICP-SFMS	24.562	25.418	29.908	25.960	28.936	29.517	27.38	5.62
11	ICP-MS	23.2	23.4	24	21.1	21.1	20.4	22.20	6.748
12	ICP-MS	26.47	26.52	27.05	22.83	24.95	25.44	25.54	4.06
13	FAAS	22.35	22.12	22.72	22.15	22.42	22.64	22.40	2.27
15	ICP-MS	25.8	24.9	25.3	25.5	25.2	25	25.28	3.09
16	ICP-OES	24.4	24.2	24.2	25.5	24.9	24.6	24.63	3.83
Results not included in the characterisation dataset									
2	INAA	30.1	29.2	29.7	29.1	29.2	29.5	29.47	1.2



European Commission

EUR 26525 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: **The certification of the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn in Bladderwrack (Fucus vesiculosus): ERM®- CD200**
Author(s): Anna Santoro, James Snell, Håkan Emteborg, Marie-France Tumba-Tshilumba, Andrea Held
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Abstract
This report describes the production of ERM-CD200, a powdered bladderwrack material (Fucus vesiculosus) certified for the mass fraction of the total content of As, Cd, Cu, Hg, Pb, Se and Zn. The material was produced following ISO Guide 34:2009 [1].
Approximately 60 kg of brown algae seaweed (bladderwrack, Fucus vesiculosus) was collected in Galway (Ireland) and processed at IRMM (Belgium) to produce a certified reference material (CRM) of seaweed powder. The produced vials containing the processed seaweed were carefully capped, sealed and stored for further certification studies.
Between-unit homogeneity was quantified as well as stability during dispatch and storage in accordance with ISO Guide 35:2006 [2]. Within-unit homogeneity was also quantified to determine the minimum sample intake.
The material was characterised by an inter-laboratory comparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [3] including uncertainty contribution related to possible heterogeneity and instability of the material as well as to the characterisation.
The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies.
The CRM is available in amber glass vials containing approximately 5 g of dried powder, placed in aluminized polythene sachet closed under a nitrogen atmosphere.
The minimum amount of sample to be used is 200 mg.
The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

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